BIOCIDAL RUBBER FOR WATER RECLAMATION SYSTEMS

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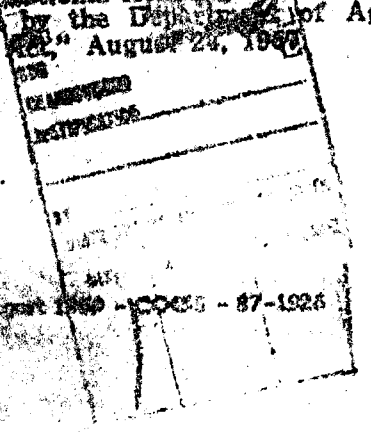

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FOREWORD

This study was conducted by Aerospace & Defense Products, A Division of The B.F. Goodrich Company, Akron, Ohio 44318, under Air Force Contract F33615-68-C-1266 in support of Project 6373, "Equipment for Life Support in Aerospace," Task 637304, "Waste Recovery and Utilization," Work Unit 007, "Biocidal Rubber for Water Reclamation Systems." The program was initiated by the Biotechnology Branch, Life Support Division, Aerospace Medical Research Laboratories,* with Mr. Albert B. Hearld as contract monitor. The study summarized in this report was performed during the period from 26 January 1968 through 30 December 1968.

Mr. G. A. Janes served as program manager and Mr. N.F. Cardarelli as principal investigator for Aerospace & Defense Products. The bacteriological investigations, both static and dynamic, were conducted by Dr. C.J. Major, Professor and Head, Department of Chemical Engineering, University of Akron, Ohio.

This technical report has been reviewed and is approved.

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Commander
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*The Biotechnology Branch and the Life Support Division were abolished in December 1968, and the Laboratories were redesignated Aerospace Medical Research Laboratory.

ABSTRACT

An assessment was made of the bactericidal efficacy of 17 biologically active elastomeric materials against 7 genera of microorganisms under static and varying dynamic conditions. A test cell and a laboratory model of a vacuum distillation water reclamation system used in the study are described. Compounds showing the greatest overall effectiveness in buffered distilled water solutions and in urine are delineated. Taste thresholds and data on rat feeding experiments using one of the biocidal agents are included.

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INTRODUCTION

Background

The attachment of sessile organisms to immersed objects in the ocean is a major problem and may lead to many undesirable fouling effects - increased drag, added weight, plugged openings, corrosion, degradation of materials, loss of structural integrity, etc. The B.F. Goodrich Company in its search for means to prevent marine fouling developed elastomeric based formulations whose efficacy is based on the slow release of repellent chemicals. The underlying principle is the incorporation of a biologically active compound into a rubber matrix so as to establish a condition of solubility in an equilibrium condition. If the agent chosen is only very slightly soluble in water, it will gradually dissolve from the rubber surface. To maintain solution equilibrium, interior molecules migrate via diffusion phenomena and replace those lost from the surface. Consequently, a continuously toxic surface and surface interface is maintained.

Tests conducted at temperate, subtropical, and tropical ocean sites have revealed biocidal rubbers to the effective against barnacles, algae, tubeworms, and other marine fouling organisms. The Department of Health, Education, and Welfare, the World Health Organization, and the Florida Department of Health have shown that the slow release of toxicants from biocidal rubbers to be effective against mosquito larvae, cercariae, and snails.

Bacteriological studies have shown that biocidal rubbers are effective against several microorganisms. The studies conducted prior to the initiation of this program were aimed at the evaluation of coatings for fabrics and sheet material for military use. Thirteen genera of microorganisms cultured in air on a number of such materials not only failed to propagate, but were actually rendered inviable by the toxic surface; that is, growth could not be induced after the microorganisms were removed from the surface. Four organotin biocides in rubber formulations were found to be effective. A number of other biocidal materials were partially effective in that some genera were destroyed while others were unaffected.[1]

[1] Cardarelli, N.F., et al, "Biocidal Rubber: Theory, Preparation and Activity," B. F. Goodrich Aerospace and Defense Products, Akron, Ohio 44318, March 1966

Program Objective

The objective of this program was to evaluate specific biocidal rubber compounds for destroying microorganisms in a water reclamation system. The system consisted of a urinal, evaporator, condenser, and a collection vessel along with connective tubing. By means of this apparatus, water is recovered from urine at low pressures and temperatures; however, the product contains excessive numbers of microorganisms rendering it unsafe for human consumption.

Biologically active rubber was viewed as a promising material for inactivating the microorganisms without substantially increasing the weight or complexity of the reclamation unit. This rubber, as embodied in U.S. Patent 3417181 (N. Cardarelli, December 17, 1968) and assigned to The B. F. Goodrich Company, was a highly efficient bacteriolytic agent under static conditions. However, the reclamation system is dynamic in nature and this program was aimed at defining the rates of bactericidal activity.

SECTION II

TECHNICAL APPROACH

This program was divided into the following efforts: the selection of microorganisms representative of those found in water reclamation systems, the selection of suitable biocidal formulations known or believed to be effective in destroying these microorganisms, and the assessment of the effectiveness of the formulations under static and dynamic conditions.

Selection of Microorganisms

The organisms used in this study were selected as being representative of some that would be found in a system used to recover water from urine that had been collected in open vessels in public rest rooms. It was desired that spherical, rod-shaped, gram-positive, and gram-negative bacteria would be included as well as at least one species of fungus. Consideration was given to prior experience with the organism, its availability, and its suitability for this effort. On the basis of these criteria, the organisms listed in Table I were selected.

TABLE I

ORGANISMS SELECTED

NOMENCLATURE	SOURCE*	TYPE	SYM.
Staphylococcus albus	AU 4	Coccus gm+	SA
Streptococcus pyogenes	ATCC 2032	Coccus gm+	SP
Escherichia coli	AU 10	Bacillus gm-	EC
Alcaligenes faecalis	AU 13	Bacillus gm-	AF
Proteus vulgaris	AU 15	Bacillus gm-	PV
Bacillus subtilis	AU 1	Bacillus gm+	BS
Hermodendrum cladosporium	WVU 756	Fungus	HC

* AU - Bacterial culture from the collection at The University of Akron
ATCC - American Type Culture Collection
WVU - From the collection at West Virginia University

In all cases, pure strains were cultured and the innoculum was identical in each experiment. Conventional aseptic transfer and test techniques normal to the art of the bacteriologist were used throughout the program.

Selection of Biocidal Formulations

Approximately 3000 biologically active rubber formulations were reviewed in order to select those believed to be effective against the organisms to be used in this program. Ten formulations which had exhibited strong bacteriolytic properties were chosen. Each had, in previous works, destroyed at least ten of thirteen test species. Organisms so used include: Staphylococcus albus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Alcaligenes faecalis, Aerobacter aerogenes, Proteus vulgaris, Bacillus subtilis, Bacillus megatheruim, Serratia marscicens, Streptococcus lactis and the fungi Aspergillus niger and Rhizopus nigricans. These formulations comprised our initial selection and are listed in Table II. (Code 350A thru 819D)

The results of the evaluation of the biocidal rubbers initially selected revealed that these formulations were not as effective against the organisms chosen as was desired for this program. Consequently, a second screening of available formulations was made. The selections are shown in Table II. (Code 324C thru 1120B)

TABLE II

SELECTION OF BIOCIDAL FORMULATIONS

CODE*	BASE ELASTOMER	TOXICANT**	PPHR*** CONCENTRATION	RELEASE RATE****
350A	Neoprene	TPTC	10.0	.
351B	Neoprene	TBTO	12.0	2.2
378E	Acrylonitrile	TBTO	5.0	3.5
419A	Neoprene	TBTAd	8.0	
419B	Neoprene	TBTR	8.0	
443A	Neoprene	TBTO	8.0	1.8
802C	Neoprene	TBTA	8.0	
802D	Neoprene	TBTF	8.0	2.5
819B	Neoprene	TBTO	6.5	
819D	Neoprene	TBTS	6.5	3.0
324C	Neoprene	TBTO	17.5	2.9
1112H	Neoprene	TBTF	20.0	2.3
1112HX	Neoprene	TBTF	15.0	3.5

TABLE II (CONTINUED)

INITIAL SELECTION OF BIOCIDAL FORMULATIONS

CODE*	BASE ELASTOMER	TOXICANT**	PPHR*** CONCENTRATION	RELEASE RATE****
1102A	Acrylonitrile (Paracril)	TBTO	11.0	4.0
B5	Natural Rubber	TBTO	8.0	
397C	Neoprene(Blue)	TBTO	6.5	
1120B	Neoprene	TBTAd	10.0	2.8

* Formulation number assigned by B.F.Goodrich

** TPTC - Triphenyltin chloride; TBTO - bis-n-(tributyltin) oxide; TBTAd - Tributyltin adipate; TBTR - Tributyltin resinate; TBTA - Tributyltin acetate; TBTF - Tributyltin fluoride; TBTS - bis-n-(tributyltin sulfide)

*** Toxicant concentration in parts per hundred parts of rubber

**** Estimated rate in micrograms/cm^2-hr

Of the above, 351B and 378E are fast release biocidal formulations whereas, 443A is a standard moderate release rate antifouling rubber. Formulation 802C is the best of the antialgae rubbers while 802D and 419A exhibited very rapid fungi kills in past tests. Both 350A and 419B have shown good bactericidal properties but are included in this program mainly to permit an assessment of TPTC and TBTR as bacterlcidal.

Formulation 324C is an extremely high loaded chloroprene presenting a high surface TBTO concentration. Formulation 1112H is a relatively slow release for a highly loaded material, while 1112HX is a very fast release highly loaded rubber. Formulation 1102A is a very fast release TBTO compound and B5 was included to provide a natural rubber based material. The chloroprenes, as a rule, impart much more "taste" to water than does a properly formulated natural rubber. Formulation 397C is a nonblack material using silica and titanium dioxide as the reinforcing element. It has an unusually rapid organotin release rate.

Static Evaluations

The effectiveness of the biocidal formulations under static conditions was observed (1) following the incubation of rubber squares inoculated with the test organisms and (2) after the incubation of distilled water, a

buffered solution, and urine, each of which contained a sample of biocidal rubber and an inoculum of test organisms. The observed growth was compared with that obtained on repeating the experiments without the biocidal rubber or with nontoxic rubber.

To provide for uniformity in reporting the growth, the scale listed below was selected and is used throughout this report.

4+	Profuse growth; similar to control.
3+	Growth obvious, little, or no extension of the initial inoculum.
2+	Growth present but spotty and generally poor.
1+	Very little growth.
-	No growth. Total lysis.

Rubber Squares

Rubber samples (nonsterilized) of each of the first eight biocidal formulations (Table II) were cut into 2 x 2-inch squares and placed in sterile petri dishes. Using aseptic techniques, the samples were inoculated with the seven test organisms (Table I) each having been cultured in Trypticase Soy Broth (TSB). Each square was inoculated with one loopful of the test organism broth and incubated for 48 hours at 37°C. Duplicate squares with A. faecalis were incubated for 48 hours at 25°C.

No growth was observed on any of the biocidal rubbers but was evident on a nontoxic rubber used as a control. The broth spots initially placed on the biocidal specimen were removed by scraping, transferred to Trypticase Soy Ager (TSA) plates, and incubated for 24 hours. No growth was observed indicating that all viable organisms had been destroyed.

The experiment was replicated three times on different days with new culture media, fresh rubber with identical results.

Distilled Water

To provide a static condition that would be representative of one found in a water reclamation system, a new test method was established. Each rubber sample was cut into small pieces* and placed in a test tube containing 5 to 10 ml of sterile distilled water to which 0.1 ml of 24-hour TSB culture of a test organism was added. All test tubes were incubated with shaking at 37°C for 48 hours except those with A. faecalis and H.

* 5 x 20 x 1.6 mm

cladosporium which were incubated at room temperature. One loopful of sample was taken from each tube and incubated on a TSA plate. The results of this experiment are given in Table III

TABLE III

GROWTH - DISTILLED WATER

	ORGANISM**						
CODE	AF	BS	HC	EC	PV	SA	SP
107A*	4+	2+	2+	-	-	-	-
350A	2+	-	-	-	-	-	-
351B	-	-	-	-	-	-	
378E	-	-	-	-	-	-	
419A	-	-	-	-	-	-	
419B	4+	3+	-	-	-	-	
443A	+	-	-	-	-	-	-
802C	+	-	-	-	-	-	-
802D	-	-	-	-	-	-	-

* A nontoxic formulation used as a control.
** See Table I for identification of organisms

Cladosporium, an unknown organism from the laboratory standpoint at Akron University, presented various culture problems. Optimum incubation temperature, media and type of culture vessel had to be determined. Eventually, we found that glucose-yeast extract was a suitable media and 48 hours at 25°C an adequate, if not optimal, incubation period. For culturing, 250-ml flasks were appropriate.

The results shown in Table III although good on the surface, are invalidated by the lack of growth on the control sample, 107A.

Buffered Solution

Inasmuch as the test using distilled water was suspect, the experiments were repeated using 0.01 M phosphate buffered water solution to provide pH and osmotic control. The procedure was the same except that 5 ml of the buffer solution was substituted for the distilled water, and H. cladosporium was not included because of culturing difficulties. A buffered solution was deemed necessary in order to determine whether cell death resulted due to the toxicant or to unsatisfactory environmental

conditions. Bacteria produced an acid by-product which is deleterious to the cell. A buffered solution will maintain a pH at which the cell will survive. Also, distilled water provides an extreme hypotonic environment for the bacteria which may result in cell lysis. A buffered solution on the other hand provides a healthy, slightly hypotonic environment for the cell. The results are presented in Table IV.

TABLE IV

GROWTH - BUFFERED SOLUTION, TEST 1

CODE	AF	BS	EC	PV	SA	SP
Control 1	4+	4+	4+	4+	4+	-
Control 2	4+	4+	4+	4+	4+	-
350A	+	-	4+	4+	4+	-
351B	-	-	-	-	-	-
378E	-	-	-	4+	-	-
419A	-	-	-	-	-	-
419B	+	-	3+	4+	4+	-
443A	-	-	-	-	-	-
802C	-	-	-	4+	-	-
802D	-	-	-	4+	-	-

Control 1 - No rubber was added to test tubes.
Control 2 - A nontoxic rubber, 107A, was used.

Since the results of Test 1 shown in Table IV indicated that the techniques being used were satisfactory and that reproducible results could be expected, three similar experiments were conducted with additional rubber formulations, 819B, 819D, B5, and 397C, added. Observations made on the first experiment are reported in Table V.

TABLE V

GROWTH - BUFFERED SOLUTION, TEST 2

CODE	AF	BS	EC	PV	SA	SP	HC
Control 1	4+	4+	4+	4+	4+	4+	4+
Control 2	4+	4+	4+	4+	4+	4+	4+
Control 3	4+	4+	4+	4+	4+	4+	4+
350A	3+	-	4+	3.5+	4+	4+	1+
351B	-	-	-	-	-	-	-
378E	-	-	-	-	-	-	-
419A	4+	-	-	4+	3+	-	2+
419B	3+	-	2+	4+	1.5+	-	-
443A	-	-	-	-	-	-	-
802C	-	-	3+	-	-	-	-
802D	-	-	1.5+	-	-	-	-
819B	4+	-	1.5+	4+	-	-	2+
819D	-	-	-	-	-	-	-
B5	-	-	-	-	-	-	-
397C	-	-	3+	-	-	-	-

Controls 1 and 2 - Same as on Table IV
Control 3 - 0.1 ml of organism inoculated on TSA plate and incubated. No rubber or buffer used.

Both the first and second experiments revealed excellent control by some of the formulations; however, in the third there was such contamination as to render data for all three suspect. For this reason, two additional experiments, Test 5 and Test 6, were conducted in replicate with formulation 324C added. The data are summarized in Table VI. As is indicated P. vulgaris is the most difficult to destroy and S. pyogenes the easiest. Most rubbers were effective against cladosporium; all were effective against S. pyogenes, which has been omitted from the table.

TABLE VI

GROWTH - BUFFERED SOLUTION, TESTS 5 AND 6

GROWTH	AF	BS	HC	EC	PV	SA
0	351B 443A 802C 397C 324C	802C 802D 819D B5 397C 324C	351B 378E 397C 419B 443A 324C 802C 802D 819D B5	351B 819D		351B 378E 443A 802C 802D 819D 324C
1+	819D B5 802D	351B 378E 443A		378E 419A B5 324C	819D B5	B5
2+	378E 819B	419A 419B 819B	350A 419A 819B	802C 802D 819B 397C	351B 324C	350A 419A 419B 819B 397C
3+	350A 419B	350A		419B 443A	378E 802D 397C	
4+	419A			350A	350A 419A 419B 443A 802C 819B	

Urine

At the conclusion of the preceeding experiments, formulations 350A, 419A, 419B, 443A, 802C, 819B, B5, and 397C were inadequate, while 819D, 378E, 802D, 324C, and 351B were considered to have passed the initial screening test. Since the formulations that failed had an organotin release rate of 1.0 $\mu g/cm^2$-hr or less, all new material, i.e., 1112H, 1112HX, 1102A, and 1102B, added for the urine tests had a faster release.

The experiments to determine the effectiveness of biocidal rubbers in urine under static conditions were conducted in the same manner as those in which the buffered solution was used, except that urine was substituted for the buffer. Rubber strips were placed in a test tube with 5 ml of fresh pooled urine to which was added 0.1 ml of a 24-hour broth culture of the test organism. The tube (contents) was incubated with shaking at 37°C for 48 hours, except AF, which was incubated at room temperature. At the end of the 48-hour period, one loopful of sample from the tube was used to inoculate a TSA plate. Table VII shows the growth observed after a 24-hour incubation.

TABLE VII

GROWTH - URINE, 24 HRS. INCUBATION
(Duplicate Averages)

CODE	AF	BS	HC	EC	PV	SA	SP
351B	-	-	-	-	-	-	-
378E	-	1+	-	-	-	-	-
802D	-	1+	-	-	-	-	-
819D	-	2.5+	-	-	-	-	-
324C	-	-	-	-	-	-	-
1102A	NR*	NR	NR	NR	-	3+	NR
1112H	NR	NR	NR	NR	-	2+	NR

* NR indicates no run.

The growth observed at 48 and 72 hours was approximately the same as shown in Table VIII; however, in control samples where no rubber was used, the growth ranged to 2+ for H. cladosporium and from 3+ to 4+ for the other six organisms.

On repeating the foregoing experiment, the results obtained were much less impressive especially with AF. Consequently, contamination was suspected so the experiment was repeated and the growth monitored at 24, 48, and 72 hours and after 5 days. Table VIII summarizes the results.

TABLE VIII

GROWTH - URINE, 24 HRS. TO 5 DAYS
(Summary)

GROWTH	AF	BS	HC	EC	PV	SA	SP
0		351B 324C 1102A 1112H 1120A		351B 378E 802D 324C 1102A 1112H 1120HX	351B 378E 802D 324C 1102A 1112H 1112HX	351B 1112HX	351B 378E 802D 1102A 1112H
1+		1112HX		819D	819D 1120B	378E 819D 324C 1102A 1112H 1120A	1112HX
2+		802D 378E				802D	819D 324C
3+		819D 1120B		1120B 1120A		1120B	1120B 1120A
4+	all		all				

Profuse growth (4+) was shown by AF and HC in urine in the presence of all formulations. This was confirmed on repeat tests. In 24-hour incubations periods, only 378E, 1102A, and 1120A had any retarding effect on AF. With HC, only 819D showed inhibition to 3+.

Time Of Kill

Since the rapidity of kill would be a factor in a water reclamation system, a determination was made of the contact time required to destroy the organisms. The growth activity of the organisms, under conditions as for tests 5 and 6, was checked at 12, 24, 48, 72, and 94 hours with triplicate checks being made at 24 and 48 hours. Data on the growth at 12 and 24 hours are included in Table IX.

TABLE IX

BACTERIOLYSIS AS A FUNCTION OF CONTACT TIME

	AF		BS		EC		PV		SA	
	Hrs.		Hrs.		Hrs.		Hrs.		Hrs.	
SAMPLE	12	24	12	24	12	24	12	24	12	24
Control I	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
Control II	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
Control III	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
350A	2.5+	2+	3+	-	4+	3+	4+	4+	4+	2+
351B	-	-	-	-	-	-	4+	?	-	-
378E	-	-	-	-	4+	-	4+	2+	-	-
419A	3+	3+	2+	2+	4+	3+	4+	4+	4+	?
419B	1+	4+	1+	-	4+	3+	4+	4+	3.5+	2.5+
463A	-	-	-	-	4+	2+	4+	4+	+	-
802C	-	-	-	-	3+	3+	1+	2+	+	-
802D	-	-	-	-	2+	2+	4+	-	+	-
819B	2+	3+	-	-	1+	1+	4+	4+	3+	3+
819D	-	-	-	-	-	-	4+	-	-	-
B5	-	1+	-	-	1+	-	4+	-	1+	-
397C	-	-	-	-	2.5+	3+	4+	4+	1.5+	-
324C	-	-	-	-	-	1+	4+	2+	?	-

? Designates questionable growth.

Experiments were performed using ground rubber in lieu of strips. In these only E. coli and S. albus were used and samples taken after 5, 15, 30, and 60 minutes, streaked on TSA plates, and incubated 48 hours at 37°C. All samples showed profuse growth. Even with 351B, which appeared to be the superior formulation, the 60-minute contact was inadequate. This was so even though grinding increased the surface area by a factor of several thousand. Repetion of the experiment gave the same results. We believe that had these tests with ground rubber been conducted with regenerated water in lieu of urine the results would have been substantially different.

Urine is a complex chemical mixture containing polysaccharides, other carbohydrates and possibly collagenous and proteinaceous material, all of which will preferentially absorb organotin biocides in contrast to the cell wall of the bacterium which is somewhat resistant. Organotins of the R_3SnX structure are extremely hydrophobic thus readily leaving water to be absorbed by almost any macromolecule present. Urine can be toxified with TBTO or TBTF but the levels must be higher than the solution equilibrium point with the organic matter present.

Effect Of Age

Another important criterion would be the age of the biocidal rubber in the reclamation system. For this program, rubber samples autoclaved 20 times were considered "old" whereas "new" samples were those that were autoclaved 6 times. Side by side comparisons of the growth at 48 hours of organisms that had been in contact with the "old" and "new" samples revealed the following:

No difference in growth in 56 comparisons.
The "new" rubber was superior by '+ to 2+ units in 8 comparisons.

Dynamic Evaluations

In a dynamic system, one can chose to place the biocidal rubber in a number of locations. One method would be to cover all surfaces thus preventing microbial growth at any point in the system. A desirable method would be to line just enough area so that the passing fluids would pick up sufficient organotin for sterilizing the entire liquid content. To investigate the feasibility of the latter method a dynamic test cell system and an all-glass vacuum distillation system were employed.

Dynamic Test Cell System

The test cell system, shown in Figure 1, consisted of a one-gallon feed solution bottle, a stopcock for regulating solution flow, a rotometer, the test cell, sample collector, and connecting tubing as required. The feed solution was distilled water with a known concentration of E. coli.

The test cell, representing a tube of a water reclamation system, was of Type 304 stainless steel, of rectangular cross section, and lined inside on two walls with 443A biocidal rubber, a neoprene with a moderate loading of TBTO which in the static evaluations had displayed the greatest overall effectiveness against the test organisms. The cell had an internal volume of 33 cc with internal dimensions of 28.3 x 2.1 x 0.56 cm. The rubber covered the 28.3 x 2.1 surfaces.

Three different flow rates, 42.0, 7.9, and 1.7 ml/minute, were used in two separate runs. Retention times of the feed solution in the cell were 0.8, 4.2, and 19.5 minutes respectively. Flow out of cell was collected and kept at 5°C, unless otherwise noted, until the analyses were conducted. Data on the two runs are presented in Table X.

TABLE X

DYNAMIC TEST CELL DATA

Flow (ml/min)	Retention (min)	E. coli/ml (average counts) Run No. 1 Feed	Run No. 1 Sample	Run No. 2 Feed	Run No. 2 Sample	Run No. 2 Sample*
42.0	0.8	5.9×10^5	4.3×10^5	6.0×10^5	5.2×10^5	5.2×10^5
7.9	4.2	5.9×10^5	4.8×10^5	6.0×10^5	4.7×10^5	2.5×10^5
1.7	19.5	5.9×10^5	3.3×10^5	6.0×10^5	1.7×10^5	0.62×10^5

* These samples from Run No. 2 were plated after 24 hours storage at 5°C.

From Figure 2 it can be seen that with a feed solution of about 6.0×10^5 E. coli/ml, the 443A rubber with retention times of 0.8 and 4.2 minutes gave only about a 50% reduction.

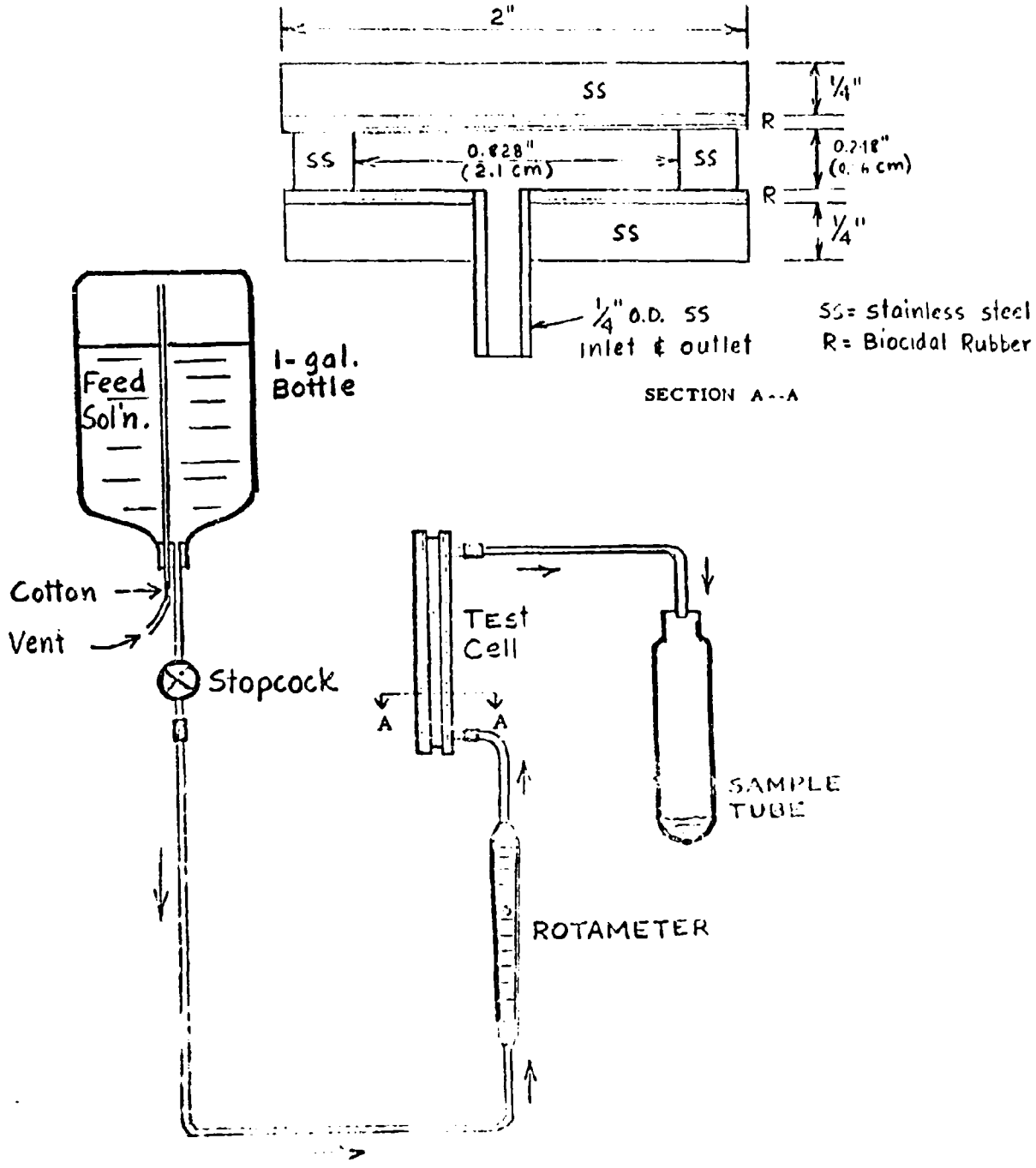

FIGURE 1. Dynamic Test Cell System

On the basis of the data obtained with the test cell system, the effluent concentration may be expressed as an exponential function of time. The equation is:

$$C = C_o E^{-kt} \qquad (1)$$

Where C = effluent concentration, organisms/ml
C_o = feed concentration
k = constant
t = time, minutes

For these runs, $C_o = 5.9 \times 10^5$ and the best value of k is 0.046. Thus, the equation 1 becomes:

$$C = 5.9 \times 10^5 E^{-0.046t} \qquad (2)$$

Equation 2 is shown as the solid line on Figure 2 where the actual data points are plotted. The data fit the exponential curve satisfactorily.

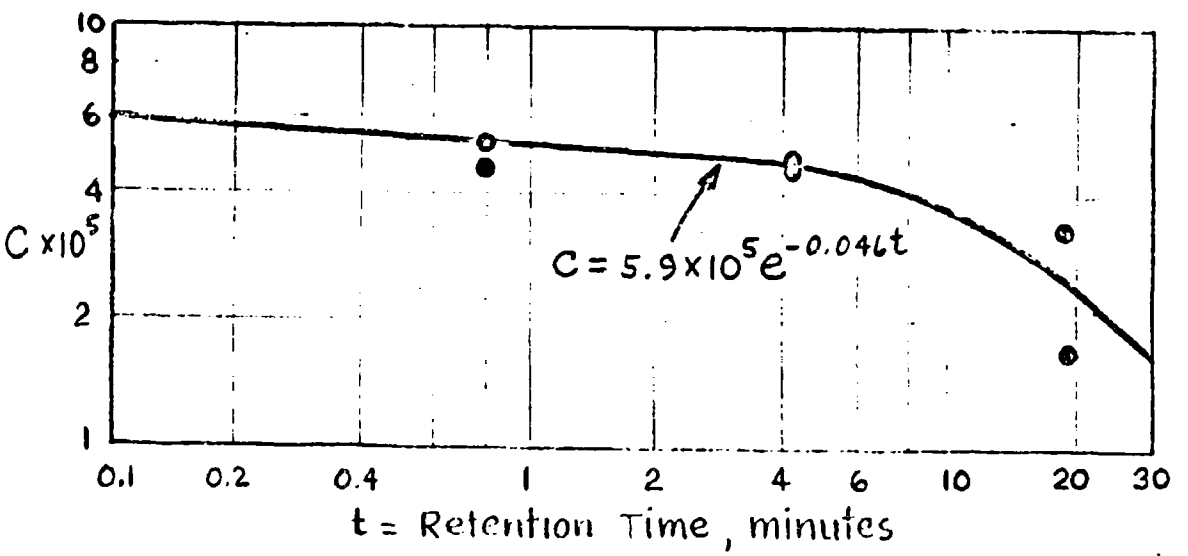

Figure 2. E. coli Count

There appears to be a residual effect of the rubber on the samples exposed for an appreciable time to the E. coli. For retention times in the dynamic cell of 4.2 and 19.5 minutes, the bacteria count is reduced by about one half if the sample is held in a refrigerator at 5°C. for 24 hours prior to analysis. For the retention time of 0.8 minute, there is no effect. This suggests that as the contact time of rubber with the bacterial solution increases, a greater amount of biocidal agent diffuses into the solution but that the biocidal agent does not kill the bacteria immediately.

Vacuum Distillation System

The all-glass vacuum distillation system, Figure 3, used a 500 ml resin kettle as the still pot. Its wide top opening made it particularly suitable for lining with biocidal rubber. All components were of Pyrex glass with ground glass connections throughout except for the vacuum lines past the product receiver. In these lines, glass tubing and rubber connections were used. The cooling water for the condenser was recirculated through a cold finger reservoir with the water temperature being maintained at about 5 to 8 C.. An ice bath was used ahead of the vacuum pump.

A magnetic stirrer-heater was used under the still pot. Two magnetic stirrer bars were used - the lower one for agitation of the water bath and the upper one to agitate the still pot contents. The column above the still was ordinarily unfilled; however, for Distillation Run No. 5 it was filled with biocidal rubber chips.

The procedure for carrying out the vacuum distillations was essentially as follows:

The whole system was first evacuated completely to remove all moisture. The ice bath and dry ice trap were then charged with ice and dry ice, respectively. The feed solution had previously been poured into a separatory funnel above the still pot. The feed solution was then transferred to the still pot, the stirrer-heater was turned on and the vacuum adjusted by means of a bleeder valve to maintain the kettle at about 104°F(40°C). Prior to actual startup of the heater, the cold finger-condenser system was turned on to maintain a cooling water circulating temperature of between 5 and 8°C.

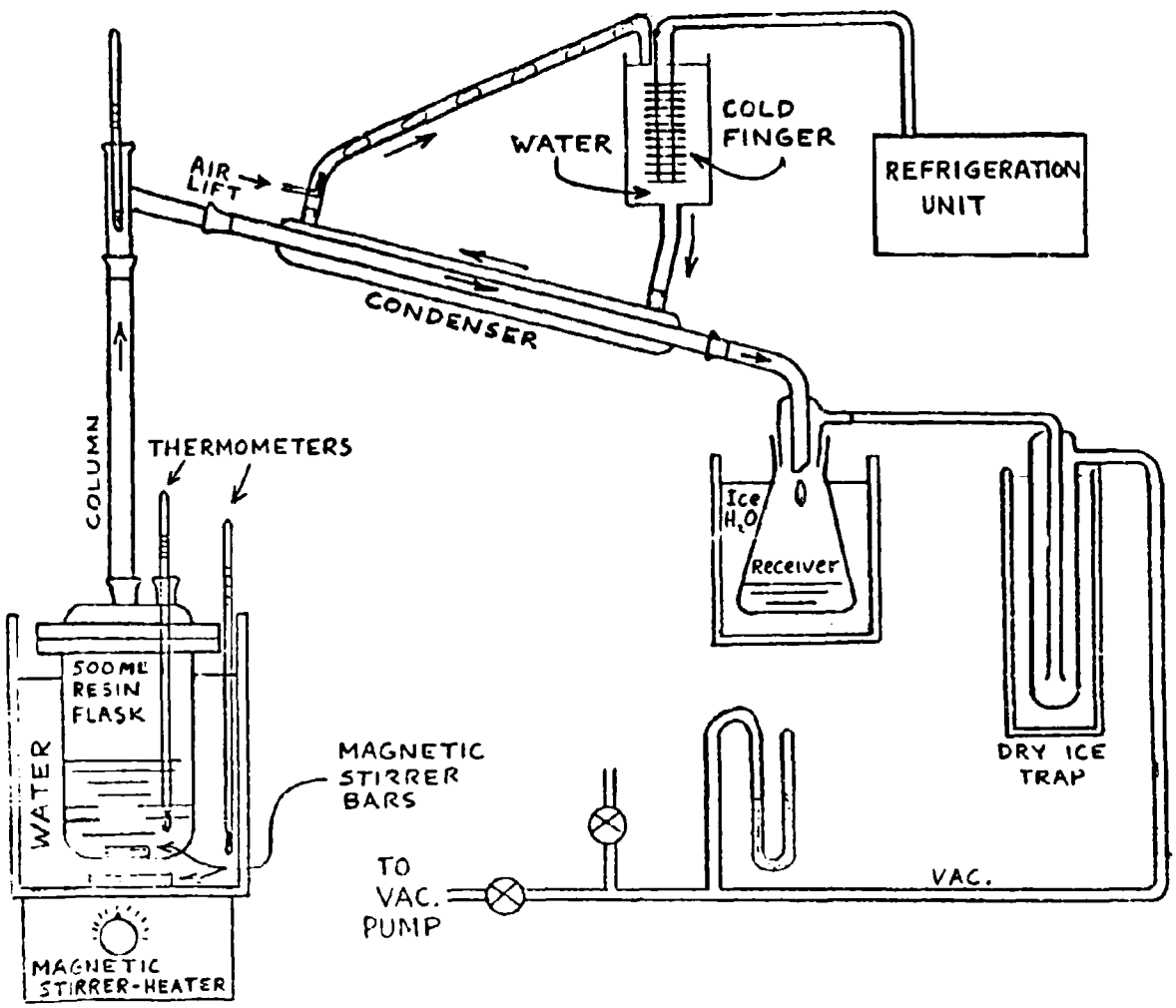

FIGURE 3. All-Glass Vacuum Distillation System

Data On Distillation Runs

Run No. 1

This run was conducted using a charge of deionized water to determine the overall material balance and the normal bacterial count to be expected in the distillate and other parts of the system. Data for the run are shown in Table XI. Loss in the system was 2 ml or 0.40% of the 500 ml charge. The 2 ml represented about 1.7% of the material (119 ml) leaving the still kettle. Bacterial counts were less than one organism per ml on 10/1 dilution of sample.

TABLE XI

VACUUM DISTILLATION - RUN NO. 1
(Deionized Water - No Biocidal Rubber)

Time	Temperatures, C			Pressure, mmH
	Bath	Kettle	Vapor Column	
11:15	24.5	21.5	23	48
11:26	30	25.4	23	54
11:32	32.5	27.8	24	54
11:37	35	30	24.5	54
11:42	38	32	25.5	54
11:48	42	35	27.5	54
11:55	45.5	38.7	37.0	54
12:30	63.8	40.8	42.5	55
12:45	end			

Recovery Data		
Receiver:		100 ml
Trap		17 ml
Left in Kettle		381 ml
Total accounted for		498 ml
Loss	=	2 ml
Total		500 ml

Bacterial Plate count on samples:	
Kettle	<1x10(No count on 10/1 dilution)
Receiver	<1x10(No count on 10/1 dilution)
Trap	<1x10(No count on 10/1 dilution)

Run No. 2

Data for this run are shown in Table XII. The feed solution contained E. coli, 710/ml.

TABLE XII

VACUUM DISTILLATION - RUN NO. 2
(E. coli, 710/ml - No Biocidal Rubber)

Time	Temperatures, C				Pressure mm Hg	E. coli/ml
	Bath	Kettle	Vapor	Condenser		
11:05	57	34	27	6	-	-
11:14	57	45	44.5	-	70	-
11:15	58	42	42	-	60	-
11:40	58	40	40	-	54	
	Sample 1 (60 ml) Distillate					< 1
12:00	62	41	42	6	53	-
12:15	63	41	41	6	53	-
	Sample 2 (105 ml) Distillate					< 1
12:51	64.5	40	41.5	4	53	
	Sample 3 (105 ml) Distillate					< 1
12:57	100 ml of Roccal added to still pot					
1:02	Sample 4 (60 ml) Distillate					< 1
1:12	Sample 5 (38 ml) Distillate					< 1
	Still pot residue					< 1
	Dry Ice Trap					< 1

Run No. 3

In this run, the feed concentration was increased to 2.2×10^6 E. coli/ml. For the 24-hour holding time, all samples showed essentially no organisms except sample 3. However, with the 48-hour holding time, 3 samples showed detectable counts. Sample 3 had 1160 organisms/ml after 48 hours holding time. Thus, even with a very high feed count, not many organisms survived. Data for the run are included in Table XIII.

TABLE XIII

VACUUM DISTILLATION - RUN NO. 3
(E. coli, 2.2×10^6/ml - No Biocidal Rubber)

Time	Temperatures, C				Pressure mm Hg	E. coli/m	
	Bath	Kettle	Vapor	Condenser		24 Hrs	48 Hrs
12:17	58	33	39	9	52	-	-
12:30	61	40	40	7	53.5	-	-
12:52	64	40	40	5	53.5	-	-
	Sample 1 (90 ml) Distillate					< 1	< 1
1:27	65	40	40	6	54	-	-
	Sample 2 (125 ml) Distillate					< 1	67
1:37	64	41	40	7	55	-	-
	Sample 3 (10 ml) Distillate					*	1160
	Still Pot Residue					< 1	6
	Dry Ice Trap					< 1	< 1

* Many pin point colonies.

Run No. 4

This was more of less a repeat of Run No. 3. The feed concentration was 3.7×10^6 E. coli/ml. The results again confirmed that not many E. coli survive a simple distillation as performed in these experiments.

The distillation system was started up at 10:50 but was shut down at 11:10 because of an apparent plug-up, the still pot temperature having reached 58°C. The system was started again at 11:30 and operated satisfactorily thereafter. Operating temperatures and pressures were comparable to those of the preceding run. Data on bacterial analyses of samples collected are included in Table XIV.

TABLE XIV

BACTERIAL ANALYSES OF SAMPLES - RUN NO. 4

Sample	E. coli/ml
Pot residue from previous batch	< 1
Feed	3.7×10^6
Sample 1 (25 ml)	17
Sample 2 (90 ml)	< 1
Pot residue at end of run	< 1

Run No. 5

In this run, chips of biocidal rubber 443A were placed in the column above the still pot and in the condenser of the distillation system. The run was started and carried out for about an hour with 110 ml of condensate being collected. The results of the analyses made are shown in Table XV. Data on the rubber chips are given in Table XVI.

TABLE XV

BACTERIAL ANALYSES OF SAMPLES - RUN NO. 5

Sample	E. coli/ml
Feed	2.35×10^6
Condensate (110 ml)	< 1
Still pot residue	2.71×10^3

The data show that the condensate contains no E. coli while the still pot residue has a count 1000 times less than the feed.

TABLE XVI

RUBBER CHIPS - RUN NO. 5

Rubber Type: No. 443A
Average Size of Each Chip: 0.555 x 0.56 x 0.216 cm
Total Number of Chips: 576
Bulk Volume of 576 Chips: 79 cc
Packed Height in Column: 31 cm
Total Surface Area: 638 cm^2 (includes all edges)
Surface Area/Chip: 1.108 cm^2

The distillations performed in the preceding 5 runs show that essentially all E. coli bacteria are killed in the vacuum distillation process without the addition of a bactericidal agent. The addition of 443A biocidal rubber yielded bacteria-free distillates. Nevertheless, we must stress that the feed solutions consisted of pure strains of E. coli in water. Urine distillations might have yielded different results.

SECTION III

TASTE AND TOXICOLOGICAL STUDIES

Taste

Formulations 351B, 324C, and 819D impart no taste discernible by man when a 5 x 20 x 0.25 mm strip is immersed in one liter of tap or distilled water for a 24-hour period at room temperature. In time; however, each will impart a slight, but characteristic, organotin taste. This occurs when the TBTO concentration in the water reaches 2 to 5 ppm. Concentrations of TBTF in excess of 7 ppm impart a taste to water.

In a feeding experiment, 10 rats were given the choice between water containing TBTO at about 30 ppm and fresh water. The preference was for the fresh water, although on occasion all rats drank from the TBTO water and several displayed an indifference to the contaminated water.

Toxicology

Rat feeding studies performed at The University of Akron prior to this program showed no gross physical changes over a 90-day period in rats that were forced to drink 351B contaminated water at the TBTO equilibrium point - approximately 30 ppm. No weight loss, nervousness, or other symptoms were observed. Upon sacrifice, no gross changes in body organs or obvious pathology existed. No histological examination of tissue was performed.

More recently the U.S. Army Environmental Health Agency has conducted toxicological examinations of various biocidal rubbers.[2] Rats and other animals were found unaffected by drinking organotin-containing water over short periods of time, i.e., up to 30 days.

[2] "Hazard Evaluation of B.F.Goodrich Biocidal Rubber No. 443A" Project No. 33-23-67/68, U.S. Environmental Hygiene Agency, Edgewood Arsenal, Maryland, November 1967.

SECTION IV

CONCLUSIONS

The rubber compounds showing greatest overall effectiveness are as follows:

Rank	In Buffered Solution	In Urine
1	819D	351B
2	324C	324C
3	802D	1112H
4	378E	1120A
5	351B	1112HX

Against specific host organisms the following order of superiority is indicated.

Organism	In Buffered Solution	In Urine
AF	324C	351B
BS	324C	324C
HC	802D	802D
EC	819D	324C
PV	819D	351B
SA	324C	351B
SP	802D	378E

In general the seven organisms will not grow upon any surface covered with any of the selected test formulations. Bacteriostasis and bacteriolysis are quite evident even though optimal conditions for growth are maintained.

In a dynamic system, flow rates must be such as to permit rubber contact with the polluted water for a time period in excess of 1 hour and less than 24 hours. In any case, to sterilize urine in a dynamic system by means of flow contact with biocidal rubber does not appear practical.

On the basis of the limited, short range rat feeding studies, the consumption of water containing 1 ppm or less of TBTO or its esters is not injurious to health, if the period involved is only several weeks in length. Lack of knowledge involving possible accumulation in the human body emphasizes the need for further study and extreme caution before permitting human ingestion.

Concentrations of less than 2 to 5 ppm TBTO and 7 ppm TBTF impart no untoward taste in tap or distilled water.

SECTION V

RECOMMENDATIONS

On the basis of the completed test program, it is recommended that in a water reclamation system:

1. The urinal be lined with compound 351B or 443A to prevent the colonial growth of organisms thereon.

2. The base of the still be covered with compound 351B.

3. The collection vessel base be lined with compound 324C.

We believe that these measures will substantially reduce bacterial contamination of the water to levels safe for human use.

Further evaluation is suggested in that it may be possible to develop a biocidal filter as a simple attachment. That is a 351B, 819D rubber mix be crumbled to vastly increase surface area, suspended between two permeable membranes, and the regenerated water passed through. Such units could be made disposable and a fresh unit easily attached. The filter would be more effective if installed between the condenser and the collection vessel.

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13. ABSTRACT

An assessment was made of the bactericidal efficacy of 17 biologically active elastomeric materials against 7 genera of microorganisms under static and varying dynamic conditions. A test cell and a laboratory model of a vacuum distillation water reclamation system used in the study are described. Compounds showing the greatest overall effectiveness in buffered distilled water solutions and in urine are delineated. Taste thresholds and data on rat feeding experiments using one of the biocidal agents are included.

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14. KEY WORDS	LINK A		LINK B		LINK C	
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Water Renovation						
Water Recovery						
Biocidal Rubber						
Urine						
Bactericide						
Fungicide						
Control Toxicant Release						
Water Treatment						
Organotin						

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